Phytochemical Analysis and Antimicrobial Activity of the Petroleum Spirit Extract of Back of the Plant Ziziphus Abyssinica (HOCCST. ex A. RICH)

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ABSTRACT

Antibiotic resistance and the evolution of new disease causing agents have become a global health concern. New source of novel drugs are therefore required for proper treatment of this diseases. Medicinal plants that are commonly used in our community could provide drugs that can combat this problems. This study aims at carry out a phytochemical analysis on the petroleum ether extract of powdered bark of *Ziziphus abyssinica* (Hoccst. ex A. Rich) and to investigate the antimicrobial activity of the plant. The phytochemical screening reveals the absence of carbohydrates (sugar, combine reducing sugar, monosaccharides, sucrose, pentoses), glycosides, tannins and alkaloids. The brine shrimp test showed that the extract was moderately toxic to the shrimps with LC_{50} value of 30.20±8.91µg/cm^3, signifying the presence of bioactive compounds. The antimicrobial screening showed that the extract was active against *Staphylococcus aureus, Streptococcus pyogen, Corynebacterium ulcanes, Salmonella typhi, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosca, Klebsiella pneumonia, Neisseria gonorheae and Candida albicans*. The extract inhibited all the pathogens thereby giving support to the use of the plant in the treatment of fever typhoid, pneumonia, cough, gonorrhea and other ailments which would be caused by the pathogens.

Keywords: *Ziziphus abyssinica*, antimicrobial screening, phytochemicals

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1. INTRODUCTION

Plants are often attacked by herbivores, insects and pathogen which may have devastating consequence on their existence and might also lead to their extinction, and hence plants produce secondary metabolites which offer protection values since they are toxic and repellant against external devices. These metabolites found in plants are responsible for medicinal properties making these plants beneficial to mankind as a source of pharmaceutical and healthcare product (Saurabh Pagare *et al*., 2015). Medicinal plants are effective source of compounds which may be antimicrobial for treatment of various infections. The use of these plants in medicine predates the introduction of antibacterial drugs into African continent. The use of plants as drugs is still relevant today as plant materials are present in or have provided model for 50% of western drugs (Ibrahim and Abubakar, 2016). Medicines obtained from plants are relatively safer, affordable and accessible than synthetic drugs.

However, the development of bacterial resistance to these existing antibiotics is a major concern. Hence, the need for continuous search for antimicrobial compounds with diverse chemical structures and novel mechanism of action against emerging infectious diseases.

Ziziphus abyssinica (Hochst. ex A. Rich) or Magarya (local Hausa name) belongs to the family Rhamnaceae that consists of small trees. It is distributed between Senegal to Cameroun eastwards to Ethiopia and south through East Africa, South and East Congo, Angola, Rhodesia and Mozambique. It is widespread in the drier parts of tropical Africa, through the central and southern provinces of the Sudan (Wickens, 1976). In Nigeria They are commonly found in Sokoto, Zaria, Yola and Lagos.

The fruit of *Ziziphus abyssinica* is taken for pains while the roots are useful for dysentery. The ash from the burnt leaf mixed with salt is used as a throat pain in tonsillitis, the hot leaf is used as a fomentation to the chest in pneumonia, and it is made into a poultice and applied to sores and treatment of snake bite (Eric *et al*., 2017). The wood is considered to be a good timber while the stem is used as hedges to exclude wild beast. In Sudan, it is used for hot poles; it is the chief fodder tree for sheep and goats in Northern Nigeria during dry season (Irvine, 1961).

Fig 1: Diagram showing Small Tree with rounded crown

Fig 2: Diagram showing Simple alternate leaves and hooked spines

Fig 3: Diagram showing Fissured back

Fig 4: Diagram showing Twig showing ripe fruits

2. MATERIALS AND METHODS

Collection and preparation of materials

The bark of Ziziphus abysinnica (Hochst. Ex A. Rich) was locally sourced collected in Zaria. The plant was properly identified and authenticated in the Herbarium of Biological Sciences of A.B.U Zaria with voucher number 527. The bark was properly cleaned to remove dust and soil and air dried for two weeks on a laboratory bench. The dried bark was pounded to powder using laboratory pestle and mortar (Nyaberi *et al*., 2010).

Extraction

80g of the plant material was packed into a soxhlet extractor and continuously extracted with 250cm^3 of petroleum spirit at 40-60^{o}C. The extraction was carried out for 62 hours after which the extract was concentrated under reduced pressure using rotary evaporator to remove any trace of solvents. The concentrated extract was transferred into a sample bottle and air dried on the laboratory bench. The dried extract was weighed on a weighing balance.

Phytochemical screening on extract from bark of *Ziziphus abysinnica* (Hochst. Ex A. Rich)

Phytochemical screening for the bark of the plant was carried out to analyze the presence of carbohydrate, glycosides, tannins and alkaloids using methods described by Tesfaye *et al*., (2016).

Brine Shrimp Lethality test

Brine shrimp (*Artemia salina*) was used to assess the bioactivity of crude extract of back of the plant *Ziziphus abysinnica* (Hochst. Ex A. Rich) using the procedure described by Quazi *et al*., (2017).

Antimicrobial screening

Bauer-Kirby method (Eva and Kalman, 2013) was used in the determination of the antimicrobial activities of the extract. The organisms were obtained from Department of Microbiology A.B.U. Teaching Hospital Zaria. All culture were checked for purity and maintained in blood agar slant. The paper disc method was used in the determination of the antimicrobial activities of the extract.

Thin-Layer Chromatography

Isolation of the compound of the Extract

Five prepared 10 x 20cm^3 glass plates were sported with the sample and developed with Petroleum ether:Chloroform (3:2) solvent system. The developed plates were air dried and viewed under ultraviolet and visible light (254nm and 366nm) respectively three bands were observed in each plate. The distances moved by the resolved bands were measured and their R_f values were determined.

3. RESULTS AND DISCUSSION

Phytochemical Screening

Table 1: Phytochemical screening of *Ziziphus abyssinica*

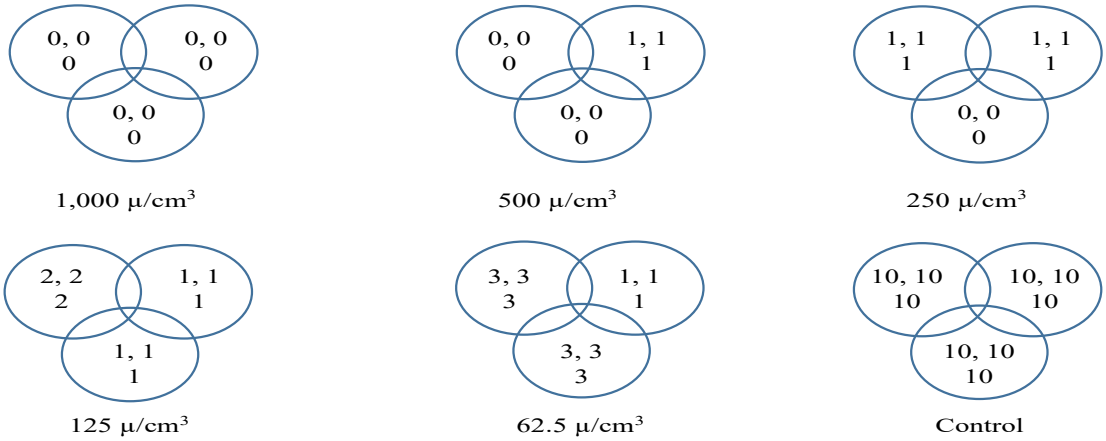
Test	Inference
Carbohydrates	-
Glycosides	-
Tannins	-
Alkaloids	-

Key: - = Absent

The extract responded negatively to the test for carbohydrates, glycosides, tannins and alkaloids as shown in table 1. The absence of these plant constituents in the extract is not surprising since petroleum ether (40-60^{o}C) is a lipophilic solvent, thus it can only dissolve non-polar constituents of an extract.

Brine shrimp Lethality bioassay

The mortality count for brine shrimp (*Artemia salina*) for the extract is shown below:



The percentage death per 30 brine shrimps (*Artemia salina*) at each concentration and control was calculated as shown in table 2 below.

Table 2: Percentage death per 30 brine shrimps

Concentration (µ/cm³)	1000	500	250	125	62.5	Control
Total shrimp per test sample	30	30	30	30	30	30
Number of survivors	0	1	2	4	7	30
Number of deaths	30	29	28	26	30	0
% Mortality;	100	97	93	87	77	0

The LC_{50} value of the extract which is the median lethal concentration that can kill 50% of the brine shrimps was calculated to be 30.20 ± 8.91 µ/cm³. This shows that the extract is toxic. The cytotoxicity test shows that the extract contains bioactive ingredients which could be of medicinal importance to man and animals.

Antimicrobial activity

Table 3: Antimicrobial activity and zone of inhibition of extract

Test organism	Activity	Zone of inhibition (mm)
Staphylococcus aureus	S	27
Streptocuccus pyogenes	S	22
Corynbacterium	S	24
Salmonella typhi	S	19
Escherichia coli	S	22
Pseudomonas aeruginosea	S	23
Klebsiella pneumonia	S	21
Neisseria gonorrhoeae	S	21
Basillus subtilis	S	21
Candida albinicans	S	19

Key: S = Sensitive, mm = millimeter

Table 4: Minimum Inhibition Concentration (MIC) of the extract against microorganisms

Test organism	$1x10^5$ mg/cm^3	$2x10^5$ mg/cm^3	$3x10^5$ mg/cm^3	$4x10^5$ mg/cm^3	$5x10^5$ mg/cm^3
Staphylococcus aureus	-	0*	+	+	+
Streptococcus pyogenes	-	-	0*	+	+
Corynebacterium ulcanes	-	-	0*	+	+
Bacillus subtilis	-	-	0*	+	+
Salmonella typhi	-	-	-	0*	+
Escherichia coli	-	-	0*	+	+
Pseudomonas aeruginosca	-	-	0*	+	+
Klebsiella pneumoneae	-	-	0*	+	+
Neisseria gonorrhea	-	-	0*	+	+
Candida albinicans	-	-	-	0*	+

Key: + = inhibition, - = no inhibition, 0* = minimum inhibition

The petroleum ether extract of the back of *Ziziphus abyssinica* (Hochst. Ex A. Rich.) was active against the various pathogens tested upon which include *staphylococcus aureus*, *Streptococcus pyogenes, Corynebacterium ulcanes, Bacillus subtilis, Salmonella typhi, Escherichia coli, Pseudomonas aeruginosca, Klebsiella pneumoneae, Neisseria gonorrhea and Candida albinicans*.

Staphylococcus aureus causes boils, monilial enteritis and other skin conditions, *Streptococcus pyogenes* causes carbuncles, tonsillitis and skin pustules; *Corynebacterium ulcanes* causes diphtheria; *Bacillus subtilis* causes pyelonephritis; *Salmonella typhi* causes typhoid fever; *Escherichia coli* causes urinary tract infection (crystitis pyelitis and pyemophiritis) *Klebsiella pneumoneae* causes pneumonia; *Neisseria gonorrhea* causes gonorrhea and neonatal conjunctivitis; *Candida albinicans* causes tonsillitis and syphilis. The activity of the extract of the back of *Ziziphus abyssinica* tends to support the traditional use of the plant in the treatment of fever, pneumonia typhoid, cough, boils, gonorrhea, etc.

Table 5: R_f values of the various components of the extract

Number on plates	Retardation factor (R_f) values		
	I	II	III
1	0.242	0.271	0.315
2	0.242	0.271	0.314
3	0.242	0.274	0.314
4	0.241	0.270	0.315
5	0.242	0.271	0.414

Distance moved by solvent = 14cm

From the separation of the various components of the extract in the petroleum ether: chloroform (3:2) solvent system, three components were obtained for each of the plates and their R_f values are given in table 5 as I, II and III for the first, second and third components respectively.According to the Brain and Turner (1975), the R_f values of a compound in a given system is a physical characteristic which is independent on the presence of other substance applied simultaneously, hence it is used in the identification of the presence of the components of an extract.

4. CONCLUSION

From the phytochemical screening carried out on the petroleum ether extract of *Ziziphus abyssinica* (Hochst. Ex A. Rich.) , carbohydrates, glycosides, tannins and alkaloids were found to be absent. Thus it has been reported that ether extract contain terpenes, steroids and fatty acid. Therefore, since some of these can cure diseases, the activity of the extract could be a result of the presence of some of these compounds. The brine shrimp test carried out showed that the extract was moderately toxic to the shrimps with LC_{50} value of 30.20 + 8.91. the antimicrobial screening showed that the extract was active against all the pathogens used, which includes *staphylococcus aureus*, *streptococcus pyogenes*, *corynebacterium ulcanes*, *Bacillus subtilis*, *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosca*, *Klebsiella pneumoneae*, *Neisseria gonorrhea* and *Candida albicans*. This implies that the extract can be used to check typhoid fever, pneumonia, gonorrhea, cough and boils. It can therefore be concluded that all the constituents present in the bark of the plants contribute to the usefulness of the plant *Ziziphus abyssinica* (Hochst. ex A. Rich.) in medicine.

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